THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY

Volume 41 No. 6 December 1997

Developmental Biology in Russia

UBC Press

Chimeric brain: theoretical and clinical aspects

SERGEY V. SAVELIEV[1], VYATCHESLAV V. LEBEDEV[2], MICHAEL B. EVGENIEV[3] and LEONID I. KOROCHKIN[3,4*]

[1,1]Institute of Human Morphology, [2]Skliphosovsky Institute of Emergency Medicine, Russian Academy of Medical Sciences, [3]Institute of Gene Biology and [4]Kol'tsov' Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia

ABSTRACT Using xeno-transplantation, interactions of neural tissues of vertebrates and insects were studied. Ventral neurogenic primordium of Notch *Drosophila melanogaster* embryos was transplanted into neural tube of amphibian and mammalian embryos with the aid of micro-hydrofeeding. Embryos of four different amphibian species, random bred mice and rats were used as graft recipients. It was concluded that there is a possibility to incorporate nerve cells of insects into the brain of vertebrates. Morphological and functional contacts can be established between the transplanted cells and host brain tissues. Transplanted *Drosophila* cells preserve their viability for a long time, so that a prolonged influence of the transplant upon the recipient can be predicted, which may be used in medical practice. A mixture of human fetal brain and Notch *Drosophila melanogaster* neural embryonic tissues were transplanted into the ventro-lateral nucleus of the thalamus of the patients of Parkinson' disease. As a result, tremor and constrained movements disappeared. Post-operation patients have been observed within 13-38 months. No side effects were noted during this time.

KEY WORDS: *brain, nerve cell differentiation, xenotransplantation, Parkinson disease*

Introduction

Despite some problems of developmental neurobiology and neurogenetics, the method of neural tissue transplantation has proved to be successful when an embryonic tissue is grafted (Giersberg, 1935; Korochkin, 1981). For instance, nerve tissue from *Spraig-Dowley* rat embryos was grafted into the anterior thalamus of adult *Wistar* rats; the donor cell survived for three months (Low *et al.*, 1983). The most interesting results were obtained using xeno-transplantation in amphibians. In this case, a genetically determined behavior of the recipients was altered according to the donor type (Giersberg, 1935; Andres and Rossler, 1977; Alexandrova and Poletaev, 1984). These results suggest possible co-existence of cells from different animal origin.

Such a possibility was demonstrated for the first time in transplantation of tissue fragments from mouse embryos into early gastrulae of toads (Barbieri *et al.*, 1983). Amphibian embryos with grafted mouse tissue fragments survived for three days; no differentiation of mouse grafted tissue was observed.

No attempts have been made to "combine" nervous systems of vertebrates and insects *in vivo*, although the compatibility of the nervous system cells of vertebrates and invertebrates was demonstrated in tissue cultures (Chen and Chen, 1971). In these experiments, it has been shown that synaptic contacts and functional relations are formed between the cells of cockroaches and birds. Our first attempts to "combine" embryonic nervous systems of insects and amphibians were unsuccessful (Saveliev *et al.*, 1989). After transplantation, cells of the *Drosophila* neural primordium were phagocytized by recipient amphibian tissue. Then, we could perform more successful experiments involving transplantation of embryonic nerve cells of *Drosophila* into neural tube of amphibian embryos (Saveliev *et al.*, 1990; Korochkin *et al.*, 1991). In these experiments, we used fragments of neural primordium of *Drosophila Notch* mutant (N84k35), located 1-3.5 (Ivanov and Sacharova, 1989). In the mutant, a whole ventral (and partially dorsal) ectoderm was transformed into nerve tissue, and its contamination by other cells was minimal (Korochkin *et al.*, 1991).

Results and Discussion

Xenograft experiments in amphibians

The results of transplantation of NDM neural primordium into neural tube of amphibian embryos proved to be different in various amphibian species. In urodeles, the compatibility between donor and recipient tissues was very low. During the first

Abbreviations used in this paper: NDM, *notch* mutant of *Drosophila melanogaster*; TRITC, tetramethylrhodamine isothiocyanate.

***Address for reprints:** Institute of Gene Biology, Vavilov Str., 34/5, 117334 Moscow, Russia. FAX: (7-095) 135 41 05. e-mail: likor@neuro.biogen.msk.su

0214-6282/97/$05.00
© UBC Press
Printed in Spain

two days after transplantation, the host embryos developed without any visible morphological changes. However, within the three following days we observed morphological and histological changes in the embryos. Amoeba-like cells with lobopodia appeared on the surface of the head ectoderm and brain. These cells migrated from embryonic tissues over the bottom of the neural plate. Histological analysis revealed that these ameboid cells were "poorly differentiated" neuroblasts, neural crest and ectoderm cells. The process of their "dedifferentiation" was accompanied by cell death. Nuclear chromatin underwent significant changes as well. The cell nuclei became rounded, the chromatin stretched in one direction and became evenly distributed within the enlarged nuclei. Then, uniformly distributed grains of chromatin were formed, often followed by chromatin compactization and finally, by cell death.

Analysis of anuran embryos showed that transplantation of *Drosophila* mutant cells into neural tube of these embryos was successful. The donor cells were visualized inside the third or fourth brain ventricle and into the host neural tissue of amphibian embryos, as well as in mammalian brain (see below) using three methods: (1) morphological examination (very small *Drosophila* cells are easily distinguished from large amphibian cells) (Fig. 1A); (2) TRITC-labeling of the graft cells (Figs. 1B and 2A); (3) *in situ* hybridization of histological sections with DNA of copy-like genetic mobile elements, which are absent in amphibians (Fig. 1C). *Drosophila* nerve cells were differentiated and formed ganglia, neuropile and proliferative zones; synaptic contacts were also observed (Fig. 2B).

It was shown that insect tissue could be located inside of any brain ventricle. However, the preferential localization occurred inside the third ventricle or around the *plexus chorioideus*, often observed during the late stages of development of the recipients. It is likely that the transplanted tissue was well adapted to a composition of the spinal cord fluid, since in our experiments no massive cell death was found.

The survival rate of transplanted tissue in anurans was similar to that in urodelians. The longest period of survival of the recipients (*Xenopus laevis* embryos) was 6 months after transplantation. *Drosophila* cells differentiated on the third and fourth days after transplantation in both, urodelians and anurans. They formed a neuropile and transformed into unipolar, bipolar, and multipolar neurons. Ganglion-like cell associations were found as well (Fig. 2C). Such structures were distributed inside the neuropile and at the margin of the donor tissue. The ganglia, consisting of the transplanted cells, contained a neuropile. Nerve cells surrounded a central region that organized the connections with the recipient brain tissue. Mix-type ganglions consisted of both, insect and amphibian nerve cells. All ganglia were connected by axon-dendrite fibers with the donor's neuropile or recipient's brain. Newformed structures, originating from *Drosophila* neurons, appeared on the internal walls of the recipient brain ventricles, as well as on the ganglia. Axons and dendrites of *Drosophila* cells penetrated the brain wall of *X. laevis*. The ependyma cells lining the ventricle walls were used as substrate for growth. Axons of *Drosophila* were translocated using the cilia of ependyma cells, which protrude into ventricular cavity (Fig. 2D).

We found morphological associations between amphibian neurons and *Drosophila* cells penetrating the amphibian brain at 50th and 180th days after transplantation. The penetration of *Drosophila* cells into brain structures was accompanied by the formation of cavities. Ganglion-like structures of mixed type were formed inside these cavities. A multipolar amphibian neuron was located in the center of this cell group, while *Drosophila* cells surrounded it. *Drosophila* nerve cells used a host neural tissue as a substrate for migration of their axons and dendrites. Similar associations also appeared inside the brain ventricles.

Melanin-containing amphibian cells were usually located in the center of such cell groups. We found a large melanin accumulation in the region of *Drosophila* cells at the 49th day after transplantation. Also formation of synaptic contacts took place at the surface of the ependyma cells. The regions of *Drosophila* cell proliferation did not depend on the age of transplanted tissue. Typically, they consisted of compact groups of undifferentiated *Drosophila* cells outside a major part of neuropile. Presence of proliferative zones probably compensated the death of *Drosophila* nerve cells, revealed six months after transplantation.

In anuran embryos the difference in growth rates between experimental and control animals was observed (Fig. 3). Development of experimental animals was accelerated. They passed through metamorphosis 10-11 days earlier and were larger than control animals. After six months of the transplantation, the difference in weight between control and experimental animals was about 1.5g.

There were also some differences in behavior reaction of experimental and control animals. To reveal these differences, we ran an experiment to compare the "surfacing reaction" in control and experimental *X. laevis* at adult stage.

The time of active attempts before surfacing and also the time of animal movements between the top and bottom platforms, as well as the number of searches between surfacing attempts, preference of the left-right exit of the top platform and the number of returns under the bottom platform after an inhale were recorded. All animals were examined daily for a week, and sometimes twice a day. Two groups of experimental frogs, e.g. carrying NDM nerve cells marked by TRITC or unmarked NDM cells, were studied. After 50 test series the frogs were anesthetized, the brains were isolated, fixed by 4% formaldehyde, and passed by light and scanning electron microscopy.

Our experiments showed that the mean value interval between surfacings was 3-4 min for 3.5-6g frogs. However, they could stay under water for 3 h without noticeable negative consequences. At the same time, the experimental animals were active only half of the time, and they were more calm than the control ones. The number of surfacing attempts was similar in both groups with a double number of exits from the labyrinth for the frogs with chimeric brains. However, we should note that this is only true for the experimental animals whose brains contained *Drosophila* cells not marked with TRITC, while the frogs with TRITC-marked cells were as active as the control ones. The latter searched for an exit from the labyrinth twice less frequently. Probably, the differences between these two experimental groups are determined by a toxic effect of TRITC because we registered a retardation in the development of the animals with transplanted cells marked by TRITC. Only after the metamorphosis they showed the same (or even faster) growth rate as control frogs.

There was also a considerable difference in the learning capacity of the animals, while each of them gained its own experience. Control frogs, just as the experimental ones, pre-

ferred the exit from the right hole of the top platform and returned to the bottom platform in more than half of the cases.

Summarizing the results of our xenotransplantation experiments in amphibians, we can conclude that *Drosophila* neural primordium transplanted into amphibian neural tube differently affects brain development in Urodele and Anura species. The compatibility of the nerve tissue of *Drosophila* and urodeles is probably low. Differentiated donor cells may synthesize peptides and other active neuro-specific substances which could cause "dedifferentiation" of recipient brain cells and surrounding tissue. Metabolic products of these nerve cells can affect the nuclear apparatus of recipient cells, because some changes in chromatin package have been found.

The transplantation of *Drosophila* neural primordium into anuran neural tube did not cause abnormalities or death of the recipients. Differentiation of transplanted *Drosophila* cells did not affect the recipient's brain morphogenesis. Connections were formed between *Drosophila* and frog cells by penetration of *Drosophila* cells into frog brain, from one side, and penetration and differentiation of amphibian neuroblasts inside *Drosophila* grafts, from the other one.

Counter neuroblast migrations are probably specific for transplantation of embryonic nerve tissue. Similar results were obtained after transplantation of brain tissue fragments obtained from 16 day old rats into the cerebellum of 1 to 2 day old rats (Jaeger and Lund, 1982).

The behavioral experiments suggest that the experimental frogs were twice more effective in escaping from the labyrinth than the control animals. A non-specific influence of *Drosophila* neurons on recipient brain can be proposed. It is possible that these differences were caused by changes in melanin metabolism (Barr *et al.*, 1983). Decreasing the number of pigment cells in the experimental animals may affect an associative brain system in frogs (a high efficiency in finding the exit from the labyrinth was not associated with increased animal movement). The level of melanin in amphibian central nervous system is high. It can be suggested that the level is determined not only by initially high pigment concentration in the egg (Kordylewski, 1983), but also by a possible metabolic influence of the transplanted *Drosophila* cells. These metabolic changes can be caused by a production of biologically active substances by proper *Drosophila* neurosecretory cells or through a stimulation of the graft cells by recipient' neuro-endocrine system.

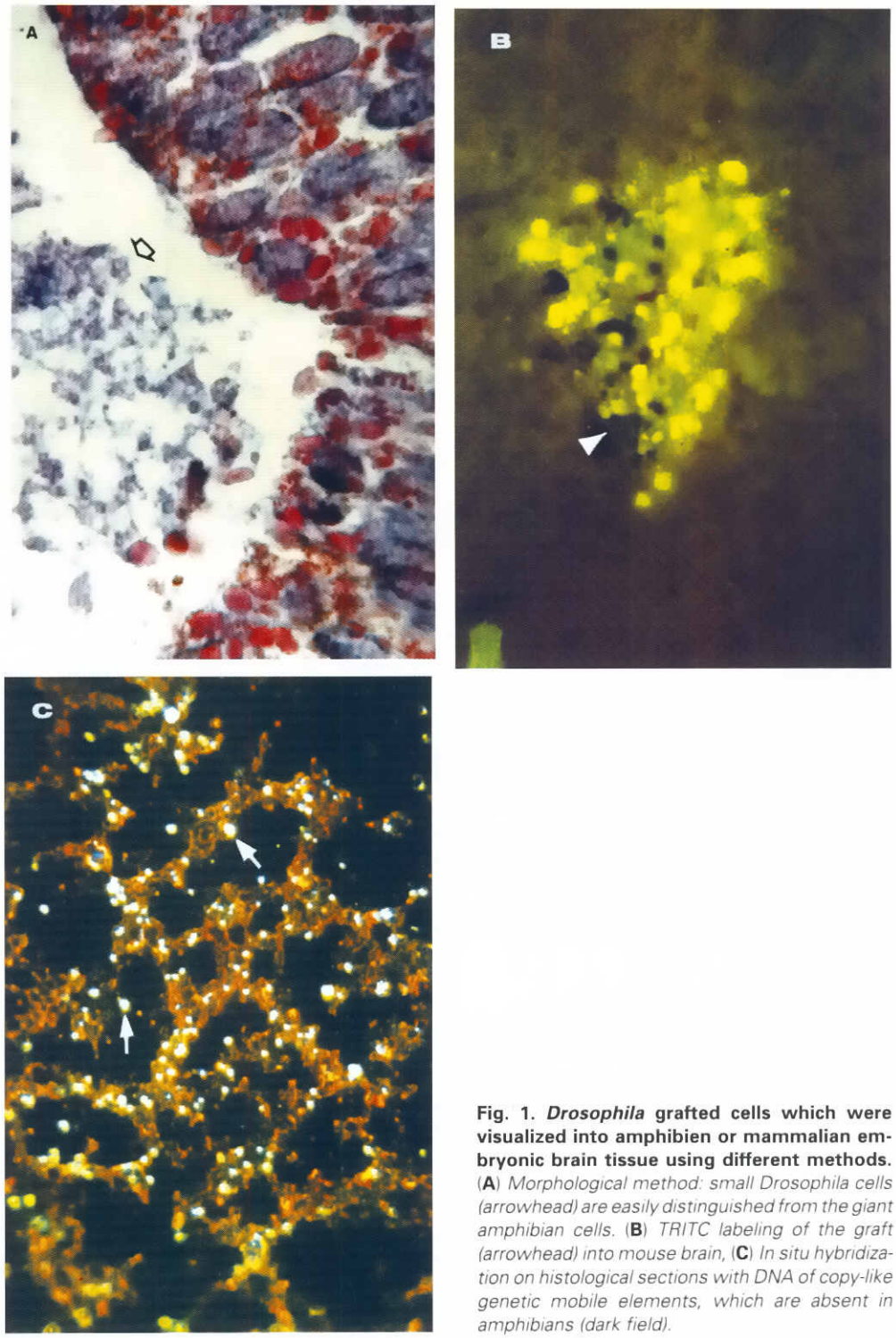

Fig. 1. *Drosophila* grafted cells which were visualized into amphibien or mammalian embryonic brain tissue using different methods. (**A**) *Morphological method: small Drosophila cells (arrowhead) are easily distinguished from the giant amphibian cells.* (**B**) *TRITC labeling of the graft (arrowhead) into mouse brain,* (**C**) *In situ hybridization on histological sections with DNA of copy-like genetic mobile elements, which are absent in amphibians (dark field).*

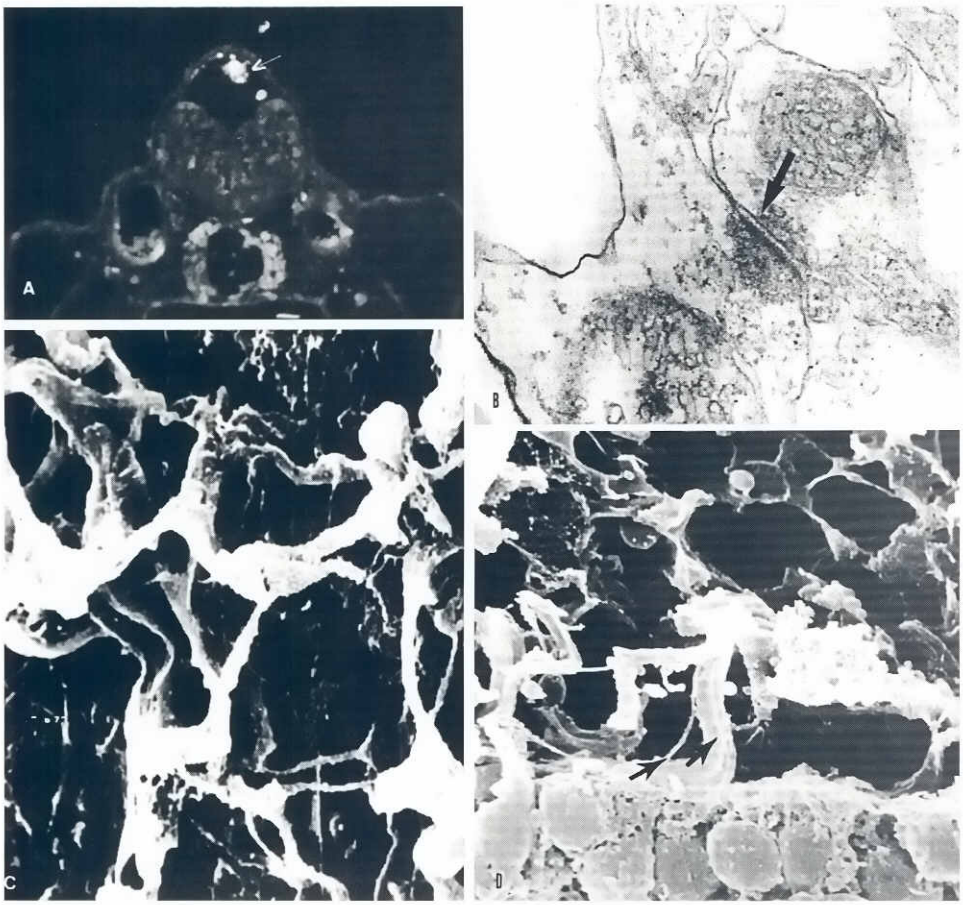

Fig. 2. *Drosophila* graft differentiation into amphibian brain tissue. (**A**) *TRITC-labeled graft (arrowhead) in the cavity of the IVth ventricle of Xenopus laevis brain.* (**B**) *Synaptic contact (arrowhead) in the graft (transmission electron microscopy).* (**C**) *Ganglion-like cell associations, which were formed into the graft (scanning electron microscopy).* (**D**) *Ependyma cells lining the ventricle walls and the axons of Drosophila nerve cells which protrude into ventricle cavity (scanning electron microscopy).*

The results indicated to a possibility of association between vertebrate and invertebrate neural tissues, interconnection of insect nerve cells with the central nervous system of amphibians and mammals could be most probable. To explain the low compatibility of neural tissue of insects and urodelians, we can assume that after differentiation of the transplanted cells, they begin to produce some peptides or neuro-active substances which may cause "dedifferentiation" of brain cells and surrounding tissues of urodelians.

The transplantation of *Drosophila* neural primordium into neural tube of anuran or mammalian embryos did not cause any abnormalities or death of the recipients. Morphological and functional contacts were established between the neurons of *Drosophila* and recipient brain. These contacts influenced the rate of development and behavior of the recipients. It is likely that morphological *in vivo* cooperation between neural cells of different animal origin may be used for predictably controlling of brain activity. There are several reasons for such a conclusion. Firstly, there is a certain degree of compatibility between insects nerve cells and brain cells of vertebrates. Secondly, morphological and functional contacts can be established between the transplanted and recipient cells. Finally, transplanted *Drosophila* cells preserve their viability for a long time, so that a prolonged influence of transplant upon the recipient is possible.

There is one additional feature of *Drosophila* neural primordium cells which make distant xenografting the most attractive method of purposeful action on the brain. Since *Drosophila* genome is well studied, we have a possibility to obtain mutant strains with nerve cells of necessary metabolic type, which may be used in medical practice.

Xenograft experiments in mammals

Analysis of mammalian brains at different periods after transplantation showed that grafted *Drosophila* cells were differentiated within 24 h, i.e., much faster than in the experiments on amphibians. The degree of morphological association between grafts and recipient' neural tissues resembled those above described (Fig. 1B). However, we observed some features specific only for mammals. Due to a fast differentiation, *Drosophila* cells did not migrate far from the site of their injection. Cell death (at 20-30%) was noted within the first 24 h after transplantation. Later, the situation was stabilized, and no further significant cell death was observed during the following two months. In mammals, we did not observe proliferation of *Drosophila* cells and formation of a cicatrix. Just as in amphibians, the donor cells stimulated vascularization of the transplant and surrounding brain structures of the recipient. The transplant was capable to form the connections with the recipient brain cells and to stimulate a growth of mammalian nerve processes into neuropile. However, undifferentiated cells did not retain the capacity for proliferation. Viable *Drosophila* cells were found both in young rats and adult mice two months after transplantation.

Parkinson disease: fetal and xenogenic neural tissue transplantations

Analysis of transplantation of human fetal tissue into the brain of patients with Parkinson disease revealed a relatively low efficiency of this approach (Olson, 1990; Madrazo *et al.*, 1991; Rosenfeld *et al.*, 1991). The use of fetal tissues isolated from the adrenal cortex and mesencephalon of human fetuses gave an evident therapeutical effect in 3-5% of cases, although the health condition of patients somewhat improved (Kupsch *et al.*, 1991; Landvall, 1991). Numerous attempts for human fetal tissue transplantation were based on a slightly modified operation scheme proposed as early as 1982 (see Olson, 1990; Landvall, 1991; Rosenfeld *et al.*, 1991). A general strategy in clinical application of transplantation of fetal tissues into the brain of patients with Parkinson disease consists of a selection of patient groups, a search for the optimal injection sites

of cell suspensions, elaboration of new transplantation techniques, use of cell cultures for transplantation, and stimulation of grafts by various growth factors (Henderson *et al.*, 1991a,b; Rosenfeld *et al.*, 1991; Iacono *et al.*, 1992; Sawle *et al.*, 1992). However, both experimental and clinical modifications of transplantation of human fetal tissues into the brain of patients with Parkinson disease have not led to expected results. The effect of fetal tissue transplantation decreased gradually within 6 to 12 post-operation months (Freed *et al.*, 1990; Quinn, 1990; Subrt *et al.*, 1991). Note that evaluation of results is very difficult due to individual features of the patients and variety of the operation schemes (Freed *et al.*, 1990; Madrazo *et al.*, 1990; Quinn, 1990; Menei *et al.*, 1991; Subrt *et al.*, 1991).

The results of more than 200 operations with human fetal tissue transplantation for treatment of Parkinson' disorders allowed us to reach two general conclusions. Firstly, the probability of stable therapeutic effect after fetal tissue transplantation is very low and does not exceed 2.5%. Secondly, modifications of already known methods of human fetal tissue transplantation apparently will not result in a significant progress of the surgical treatment of the disease. These conclusions are borne out by the attempts of different authors to provoke an interest of scientists and clinicists to create genetically modified cells with determined properties, to stimulate a differentiation of the transplanted cells by different growth factors, to use the so-called "dophaminergic transplants" obtained from cultivated *in vitro* human fetal tissues, and to test a possibility of animal brain tissue transplantation into the brain of humans (Olson, 1990; Staal *et al.*, 1990; Madrazo *et al.*, 1991). We concluded that mixed fetal-xenogenic transplantation may increase the efficiency of such a treatment. Here we present the first results on fetal and xenogenic tissue transplantation in the patients with Parkinson disease being upon observation for 6-12 post-operation months.

The first patient was a 43 year old woman with bilateral rigid-tremor form of Parkinson disease at stage 3-4. The woman could not move and serve herself. She suffered from the disease for eight years. During operation, the right-side cryotomy of the thalamic ventro-lateral nucleus was made. The cryotomic topology was based on the fact that the left extremities were firstly affected. Within 10 min after the cryotomy, 2 ml of a mixture of human fetal and xenogenic tissues (1000:2) was injected. Tremor and rigidity disappeared already on the operating table. After the operation, the patient was able to move and serve herself. Nine months later, a similar cryotomy of the left ventro-lateral nucleus of thalamus was performed. No signs of disease relapse or side effects of the transplantation were observed during 13-38 post-operation months.

The second patient was a 49 year old man with bilateral rigid-tremor form of the disease (stage 3-4) that had appeared seven years ago. Before transplantation of human fetal and xenogenic tissues, he had been operated twice. Firstly, the cryotomy of the thalamic left ventro-lateral nucleus was performed. The effect of this operation lasted for one month. Then, health condition of the patient deteriorated to the pre-operative state. Three years later, a similar operation was repeated but it did not change the patient health conditions. Six months later, the patient was subjected to transplantation of the cell mixture in accordance with above described scheme. Examination of the patient after the operation showed the absence of tremor and constrained movements. During 32 months of observation we did not reveal any relapse or side effects.

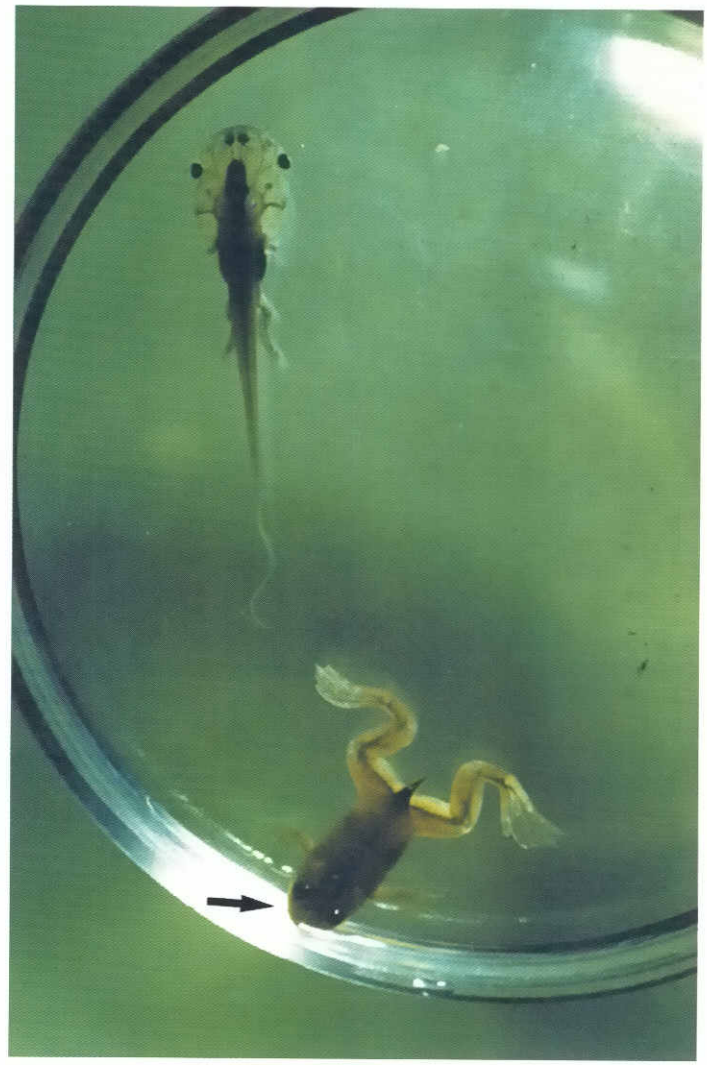

Fig. 3. Control and experimental *X. laevis* after transplantation. *Development was accelerated in experimental animals (arrowhead). They passed through metamorphosis by 10-11 days earlier and were larger than the control animals.*

The third patient was a 60 year old woman suffering from the bilateral rigid-tremor form of Parkinson disease for 10 years. In the course of operation, the thalamic ventro-lateral nucleus was cryo-destroyed on the left side of the brain without injection of the mixture of neural cells. At the same time, fetal and xenogenic tissue mixture was injected into the right ventro-lateral nucleus of thalamus without its cryo-destruction. Tremor and constrained movements on the right side disappeared directly after cryo-destruction of the left thalamic ventro-lateral nucleus. The effect of cryo-destruction weakened within four months after the operation. On the left side of the body, all specific signs of disease were preserved at the moment of transplantation. However, two months later, the tremor and rigidity on the left side disappeared. Thus, the positive effect of transplantation manifested itself only after two first post-operation months, and it was preserved within seven months after transplantation. These results indicate that it is possible to use transplantation of human fetal and xenogenic tissues for treatment of Parkinson' disease.

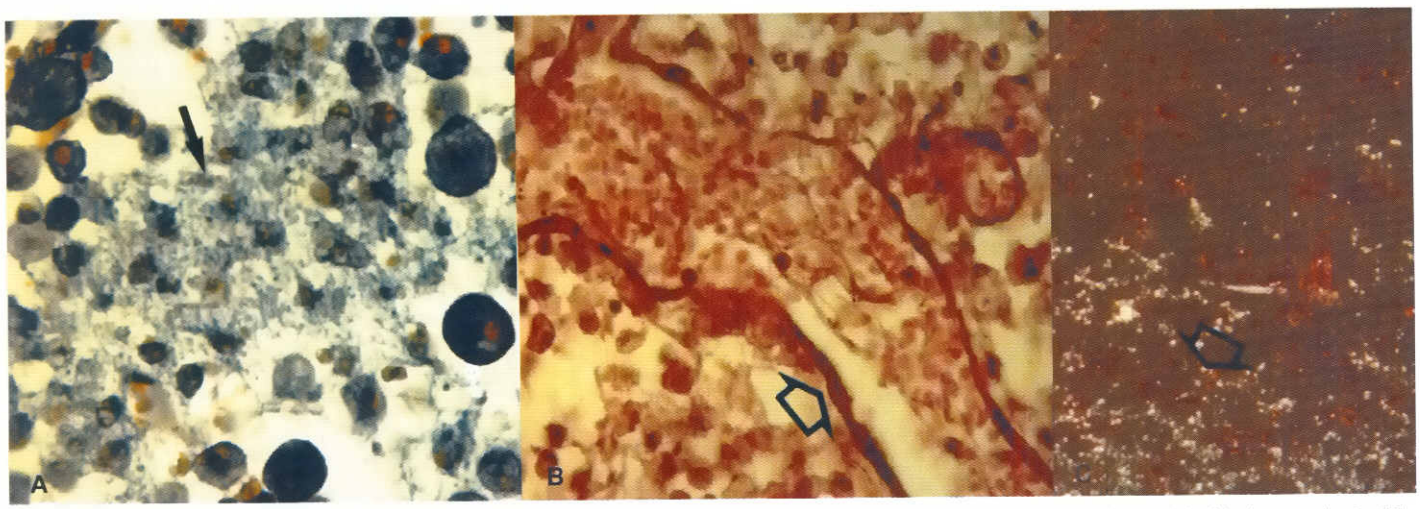

Fig. 4. *Drosophila* graft differentiation into human brain. (**A**) *Drosophila nerve cells and neuropil (arrowhead) formed inside human brain.* (**B**) *Vascularization (arrowhead) of transplantation region of human brain.* (**C**) *Drosophila cells (arrowhead) inside human brain. In situ hybridization with DNA probe of copy-like element of D. melanogaster (dark field).*

Two operation strategies used were characterized by different application of transplantation injections. A combination of cryo-destruction with cell transplantation had an immediate positive effect. However, in this case the fast improvement of the patient's health conditions was due to cryo-destruction rather than to transplantation of fetal and xenogenic tissues. After the transplantation, positive therapeutic effect manifested itself only on 1.5-2 months. We suggest that during that time transplanted human brain fetal tissue can start to differentiate under influence of the xenograft, and their common "activity" began to affect positively the patient' health conditions.

We also had the possibility of studying autopsy material obtained from one patient (with Parkinson disease at 3-4 stages) treated in accordance to our technique, whose death had been caused by non-neural disorders (Figs. 4A,C). There were no histological signs of the cicatrix between a host brain and transplant (Fig. 4A). Transplanted tissue consisted of large multipolar neurons whose size was 2-5 times larger than that of thalamic cells. Neurons and *Drosophila* cells formed the contacts, mainly by collateral axons. In this area, there were a lot of *Drosophila* cells, which formed the neurogile characteristic for insect neural tissue (Fig. 4A). However, this neurogile included fetal human neurons. Neuronal fibers of the host and transplanted neurons penetrated one to another. Under low magnification, bundles of neuronal fibers connected host brain and transplanted tissue. Bundle size suggested that the fibers consisted of 50 to 200 neuronal processes. Processes of these multipolar neurons, similar to pyramidal cortex neurons, spread both in the host brain and in transplanted tissue.

Capillaries and arterioles penetrated between transplanted and host tissue (Fig. 4B). Capillaries disposed in parallel with neuronal fibers. Some of the transplanted neurons disposed on the surface of endothelial capillary cells. Neurons grouped around capillaries were characterized by short processes and part of them penetrated inside capillaries. However, capillaries had no macrophages and leukocytes inside them. Neurons without long processes, which were disposed near the capillaries, had all morphological signs typical for secretory cells.

These data are confirmed by the results of autopsy examination of the patients treated by fetal tissue transplantation (Zabek *et al.*, 1992). It has been shown that, within five post-transplantation weeks, dophaminergic cells injected into the head of caudate nucleus survived, differentiated and displayed the positive tyrosin hydroxilase immunohistochemical reaction. Using the transplantation therapy, it is logical to observe the same time-retention in development of positive therapeutic effects, as remote results which are determined by differentiation of the injected tissue. However, the data obtained suggest that a major part of the effects observed was caused by surgery rather than by cell transplantation.

The results obtained allowed us to reveal both positive and negative aspects of transplantation of fetal and xenogenic tissues. The advantage of this approach is the possibility to treat Parkinson disease at advanced stages which usually relapse after the stereotaxic standard interference. The most important result is the possibility to use a mixture of human fetal and xenogenic tissues without cryo-destruction of the brain. The reduction of brain injuries may be very important for a recovery of brain functions under stimulating influence of fetal-xenograft. The use of a mixture of cells obtained from different animal species is a successful combination of undifferentiated structural elements of human embryonic nervous tissue and growth factors secreted by transplanted cells. Virtually, the use of neural primordia of different mutants and strains of invertebrates makes accessible an application of already obtained cell lines with known properties. However, some problems still remain. One of these is connected with the difficulty of isolating sufficient amounts of neural embryonic cells and their cryo-preservation.

Summarizing our xeno-transplantation experiments in amphibia and mammals, we can conclude that *Drosophila* neural anlage transplanted into the brain influences on its development and does not cause any abnormalities or neuron death in recipients. Morphological contacts are formed between the neurons of donor and recipient neural tissues. These results suggest that there is a possibility of association between the nervous system of vertebrate and invertebrate animals. It is important that *Drosophila*

transplanted cells preserve their viability for a long time, so that a prolonged influence of transplants upon the recipient could be possible. It is attractive to introduce in recipient organism, using *Drosophila* cells, a certain mammalian genes, which are important for nerve cell differentiation, under the controlled promoter.

Materials and Methods

Transplantation in amphibians

As donor tissue, we used the ventral neurogenic primordium of *Notch* mutant embryos of *D. melanogaster* (NDM). NDM was produced and selected in our laboratory; it is characterized by neural transformation of ventral ectoderm; N84k35 mutant embryos were used in our experiments. After mechanical removal of chorion, the embryos were isolated at stage 9 (Bowness, 1975), dissected with needles, and neural primordium pieces were isolated. Groups of cells so obtained were collected into micromanipulator-mounted glass capillaries (l00-200 μ of diameter, MM-1 micromanipulator, Russia) and injected into neural tube of amphibian embryos by micro-hydrofeeding.

Hynobius keyserlingii, *Pleurodeles waltlii*, *Xenopus laevis* and *Rana arvalis* embryos were used as recipients. Transplantation was carried out immediately following the neural tube closure. Recipients were fixed within hours (2-20) or days (2, 3, 5, 14, 49, 50 and 180) after the operation. For light microscopy, the material was fixed in Carnoy or Bouin solutions, or in 4% formaldehyde . The amphibian heads were embedded in paraffin wax. Serial sections (10 μm) were attached to slides and stained by Mallory or haematoxylin-eosin. The sections were impregnated with 1% $AgNO_3$, prepared in a 10% solution of fresh ovalbumin with ammonium chloride, for 20 h and then in 15% $AgNO_3$ solution for 24 h. The sections were treated with 1% hydroquinone in 10% Na_2SO_3 for 5 min then, in $Na_2S_2O_3$ for 5 min, dehydrated and mounted in Canada' balsam.

For scanning electronic microscopy, amphibian brains were fixed by glutaraldehyde, lyophilized, and floated with gold. The samples were examined under a Jeol 100CXII electron microscope (Japan). To identify the grafts, *Drosophila* cells were marked with Tetramethylrhodamine isothiocyanate (TRITC) and fluorescein-dextran (Gimlich and Broun, 1985). To identify *Drosophila* nerve cells inside the amphibian brain, we used *in situ* hybridization on sections with DNA of the donor copy-like genetic mobile elements. In these experiments, the *Drosophila* mdg-4 element, which amphibians lack, was used (Vashakidze *et al.*, 1987).

The test for behavioral analysis was based on physical necessity of adult frogs to come up to the water surface for respiration. As it was shown in 50 preliminary observations, time-interval between two respirations was 3-4 min for 3.5-6.0g frogs. Taking these data into account, a vertical water labyrinth was constructed. Its walls were made of transparent plastic, while the lower area was made of frosted glass. The lower area had a single exit in the center while the upper one had two exits at both sides. The diameter of each exit was 6 cm. We placed the animals under the lower area and observed them during a 30 min cycle as they looked for the exit from the labyrinth. Altogether, we carried out 90 cycles of observations recording the total time of active search, number of search periods and surfacing. At the end of observations, the animals were narcotized, and their brains were isolated followed by histological analysis. Altogether, over 200 transplant experiments were performed.

Transplantation in the patients with Parkinson disease

Human fetal tissues (ventral part of mesencephalon and basal ganglions of telencephalon) obtained from abortions between 14 and 18 week were used. The isolated tissues had been preserved until operation in liquid nitrogen for 1-3 days. It is known that such a procedure does not change the morphological structure and biochemical properties of tissues (Nadvornik *et al.*, 1991).

For xenotransplantation we used the *Notch D. melanogaster* neurogenic primordium. Before the operation, fetal human brain and *Drosophila* neurogenic tissues were mixed as described (Saveliev *et al.*, 1990; Korochkin *et al.*, 1991). Weight proportions between fetal human tissue and *Drosophila* xenotransplantat were 1000:20 to 1000:1 correspondingly. Cell mixture was injected into ventro-lateral nucleus of the thalamus through a micro-canule mounted on a stereotaxic device in a special cavity preliminary prepared in the thalamus. The cavity was made by the method of brain tissue translocation using a small balloon with changeable geometry. This device was mounted on the stereotaxic apparatus and introduced into the brain through the micro-canule. During operation, the injecting of tissue suspension was controlled with the aid of radiography.

Autopsy case

The mixture of human fetal brain tissues and *Notch D. melanogaster* neural primordia has been transplanted into sub-cortical region of the patient with ischemic brain syndrome and into thalamus ventro-lateral nucleus of the patients with Parkinson disease at the 3rd-4th stages of the pathologic process. Death of the patients was caused by non-neural disorders. Autopsy (time after transplantation, 1-1.5 months) of patient' brain tissue was performed and studied.

Acknowledgements

This work was supported in a part by the Russian Foundation of Fundamental Investigations.

References

ALEXANDROVA, M. and POLETAEV, L. (1984). Xenotransplantation of brain in frog and changes of behavior in animals. *Proc. USSR Acad. Sci. (Dokl. Akad. Nauk SSSR) 274:* 711-715.

ANDRES, G. and ROSSLER, E. (1977). Transplantation von Verhalten. *Iumschau. 74:* 144-174.

BARBIERI, F., CASTRO, N. and MICELI, D. (1983). Compatibility and nutritional dependence of mouse egg cylinder fragments associated with amphibian embryonic tissues. *Reprod. Nutr. Dev. 23:*599-606.

BARR, F., SALOMA, J. and BUCHELE, M. (1983). Melanin: the organizing molecule. *Med. Hypotheses 11:* 91-92.

BOWNES, M. (1975). A photographic study of development in the living embryo of *Drosophila melanogaster. J. Embryol. Exp. Morphol. 33:* 789-801.

CHEN, J. and CHEN, M. (1971). Combined cultures of avian and insect embryonic nervous system. *Arch. Ital. Biol. 109:* 218-235.

FREED, C.R., BREEZE, R.E., ROSENBERG, N.L., and SCHNECK, S.A. (1990). Therapeutic effects of human fetal dopamine cells transplanted in a patient with Parkinson's disease. *Prog. Brain Res. 82:* 715-721.

GIERSBERG, H. (1935). Gehirntransplantationen bei Amphibien. *Zool. Anz. 8:* 160-168.

GIMLICH, R. and BROUN, J. (1985). Improved fluorescent compounds for tracing cell lineage. *Dev. Biol. 109:* 509-514.

HENDERSON, B.T., CLOUGH, C.G., HUGHES, R.C., HITCHCOCK, E.R. and KENNY, B.G. (1991a). Implantation of human fetal ventral mesencephalon to the right caudate nucleus in advanced Parkinson's disease. *Arch. Neurol. 48:* 822-827.

HENDERSON, B.T., KENNY, B.G., HITCHCOCK, E.R., HUGHES, R.R.C. and CLOUGH, C.G. (1991b). A comparative evaluation of clinical rating scales and quantitative measurements in assessment pre- and post-striatum implantation of human fetal mesencephalon in Parkinson's disease. *Acta. Neurochir. (Suppl.) (Wien). 52:* 48-50.

IACONO, R.F., TANG, Z.S., MAZZIOTTA, J.C., GRAFTON, S. and HOEHN, M. (1992). Bilateral fetal grafts for Parkinson's disease. *Stereotact. Funct. Neurosurg. 58:* 84-87.

IVANOV, A. and SACHAROVA, N. (1989). Embryonic morphogenetic effects of mutation *Notch 84k35* of *Drosophila melanogaster. Ontogenez (Sov. J. Dev. Biol.) 20:* 280-286.

JAEGER, C. and LUND, R. (1982). Influence of grafted glia cells and host mossy fibers on anormalously migrated host granule cells surviving in cortical transplants. *Neuroscience. 7:* 3069-3076.

KORDYLEWSKI, L. (1983). Experimental evidence for the accumulation of egg pigment in the brain cavities of *Xenopus* tadpoles. *J. Exp. Zool. 227: 93-96.*

KOROCHKIN, L. (1981). *Gene Interactions in Development.* Springer-Verlag, Berlin-Heidelberg-N.Y.

KOROCHKIN, L., SAVELIEV, S., IVANOV, A, EVGENIEV, M., BESSOVA, N. and GULIMOVA, V. (1991). Nerve cells of *Drosophila Notch* mutant are differentiated inside amphibian brain: a new approach for the analysis of genetic control of nerve cell differentiation. *Genetica (Netherl.) 85:* 23-24.

KUPSCH, A., SAUERER, H. and OERTEL, W.H. (1991). Transplantation of dopamine producing nerve cells: a new therapy in idiopathic Parkinson syndrome? *Nervenarzt. 62:* 80-91.

LANDVALL, O. (1991). Prospects of transplantation in human neurodegenerative diseases. *Eur. Neurol. 31.* (Suppl.) *1:* 17-27.

LOW, W., LEWIS, P. and TERRIBUNK, S. (1983). Embryonic neural transplants across a major histocompatibility barrier: survival and specificity of innervation. *Brain Res. 262:* 328-333.

MADRAZO, I., FRANCO-BOURLAND, R., AGUILERA, M., OSTROSKY-SOLIS, F., CUEVAS, C., CASTREJON, H. and MADRAZO, M. (1991). Development of human neural transplantation. *Neurosurgery. 29:* 165-176.

MADRAZO, I., FRANCO-BOURLAND, R. and OSTROSKY-SOLIS, F. (1990). Human fetal homograft to the nigrostriatal system for the treatment of Parkinson's disease. *Arch. Invest. Med. (Mex) 21(2):* 210-207.

MENEI, P., GUY, G. and POUPLARD-BARTHELAIX, A. (1991). Intracerebral grafts in Parkinson's disease. *Presse Med. 20:* 513-517.

NADVORNIK, P., ROZHOLD, O., KOLARIK, J., ONDRA, P., ZAVODNY, P. and HLUSTIK, P. (1991). Histological and biochemical characteristics of cryoprotected embryonic nervous tissue for transplantation purpose. *Acta. Univ. Palacki. Olomuc. Fac. Med. 130:* 125-129.

OLSON, L. (1990). Grafts and growth factors in CNS. Basic science with clinical promise. *Stereotact. Funct. Neurosurg. 55:* 250-267.

QUINN, N.P. (1990). The clinical application of cell grafting techniques in patients with Parkinson's disease. *Prog. Brain Res. 82:* 619-625.

ROSENFELD, J.V., KILPATRICK, T.J. and BARTLETT, P.F. (1991). Neural transplantation for Parkinson's disease: a critical appraisal. *Aust. NZ. J. Med. 21:* 477-484.

SAVELIEV. S., KOROCHKIN, L., IVANOV, A., EVGENIEV, M. and BESSOVA, N. (1989). Transplantation of *Drosophila* cells into amphibian embryos. *Proc. USSR Acad. Sci. (Dokl. Akad. Nauk SSSR) 305:* 1239-1241.

SAVELIEV, S., KOROCHKIN, L., IVANOV, A., EVGENIEV, M., BESSOVA, N. and GULIMOVA V. (1990). Transplantation of neural primordium of *Drosophila* into neural tube of amphibian embryos. *Proc. USSR Acad. Sci. (Dokl. Akad. Nauk SSSR) 313:* 1491-1493.

SAWLE, G.V., BLOOMFIELD, P.M., BJORKLUND, A., BROOKS, D.J., BRUNDIN, P., LEENDERS, K.L., LINDVALL, O., MARSDEN, C.D. and REHNCRONA, S. (1992). Transplantation of fetal dopamine neurons in Parkinson's disease: PET [18F]6-L-fluorodopa studies in two patients with putaminal implants. *Ann. Neurol. 31:* 166-173.

STAAL, M.J., HOGEN ESCH, R.I., TOMASINI, R. and KRAYENBRINK, R. (1990). Qualitative and quantitative examination of rat and human fetal dopaminergic grafts. *Stereotact. Funct. Neurosurg 54-55:* 290-296.

SUBRT, O., TICHY, M., VLADYKA, V. and HURT, K. (1991). Grafting of fetal dopamine neurons on Parkinson's disease. The Czech experience with severe akinetic patients. *Acta Neurochir. Suppl. (Wien)52:* 51-53.

VASHAKIDZE, R., ZELENTSOVA, E., EVGENIEV, M. and KOROCHKIN, L. (1987). Repeating sequences dispersed through genome of *Drosophila* species of the *virilis* group. *Mol. Biol. (Mosk) 21:* 928-935.

ZABEK, M., MAZUROWSKI, W., DYMECKI, J., STELMACHOW, J., GAWUR, B., TRAUTSOLT, W. and ZAWADA, E. (1992). Transplantation of fetal dopaminergic cells in Parkinson disease. *Neurol. Neurochir. Pol. (Suppl. 1):* 13-19.